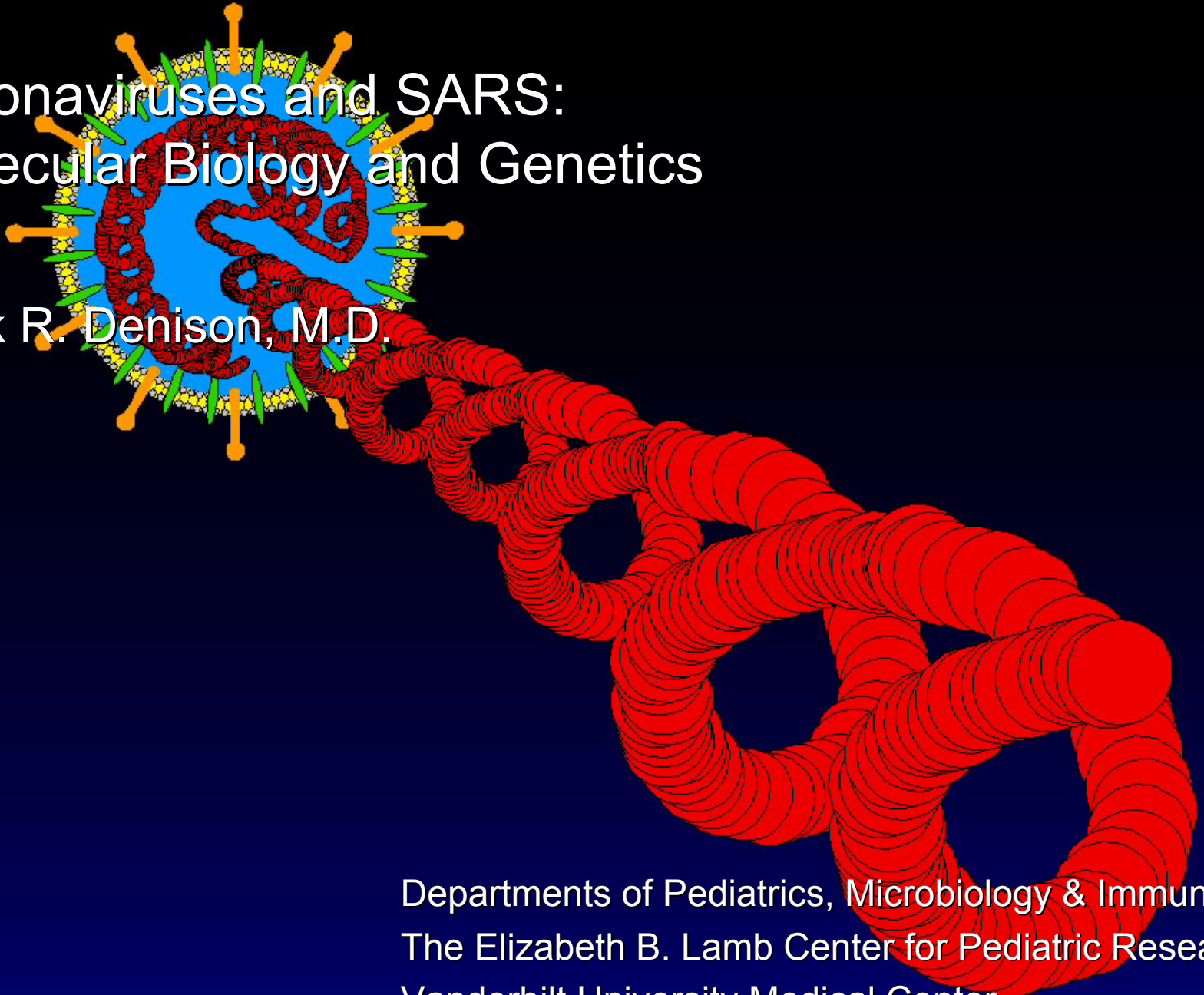


Coronaviruses and SARS: Molecular Biology and Genetics

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The Elizabeth B. Lamb Center for Pediatric Research
Vanderbilt University Medical Center



Objectives

- Review pandemic potential of coronaviruses and associated features of biology and molecular biology
- Describe coronavirus life cycle and replication
- Review molecular biology and genetics of coronaviruses
- Summarize research priorities in SARS-CoV molecular biology and genetics

SARS, Public Health, and Research

SARS - CoV

Pandemic Potential

High mortality

Worldwide spread

Naive population

Public Health Response

Coordinated action

Case Finding

Isolation

Research

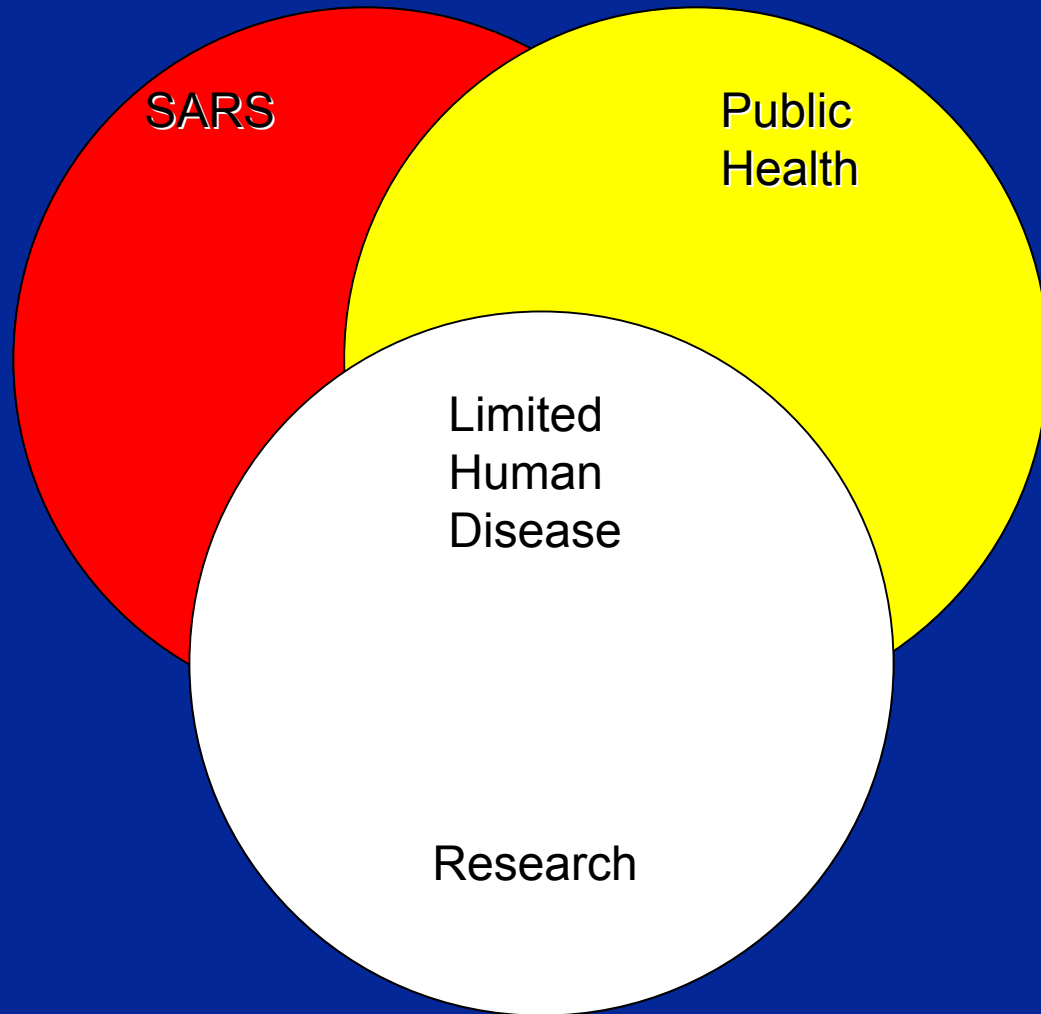
Virology

Immunology / Vaccines

Molecular Biology

Animal Studies

SARS, Public Health, and Research

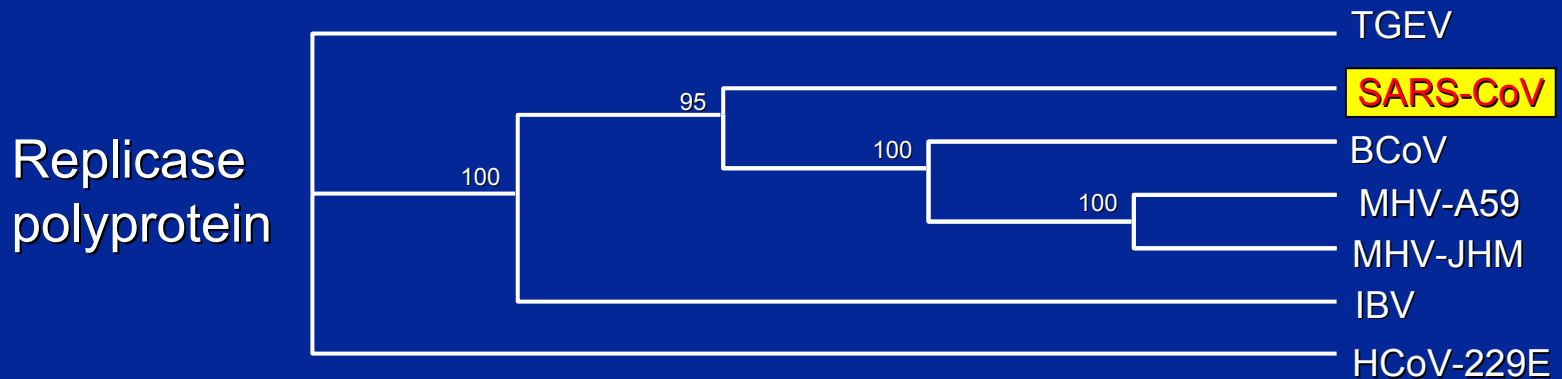
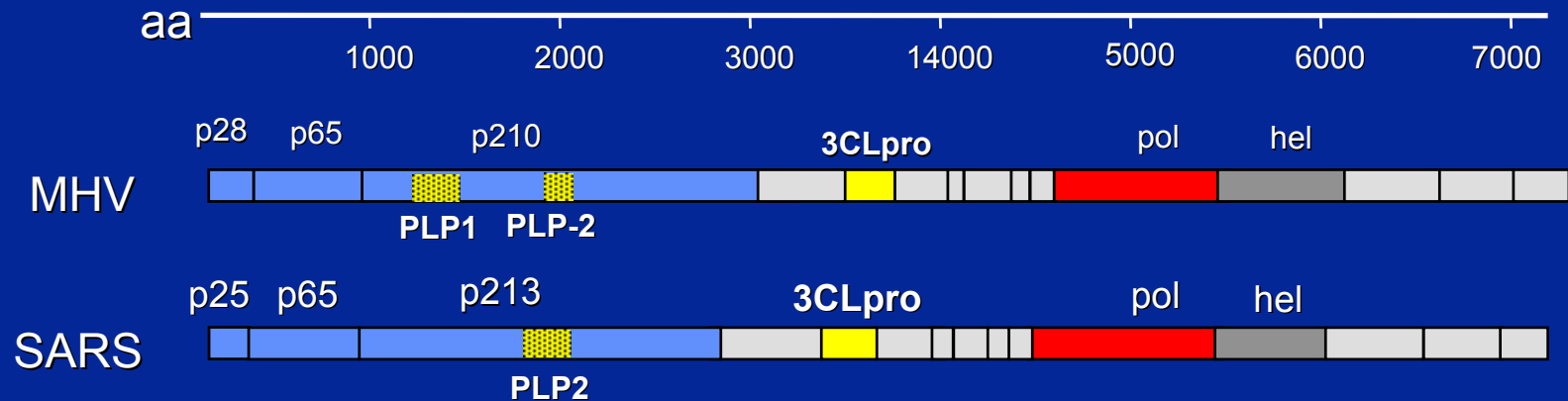


SARS, Public Health, and Research



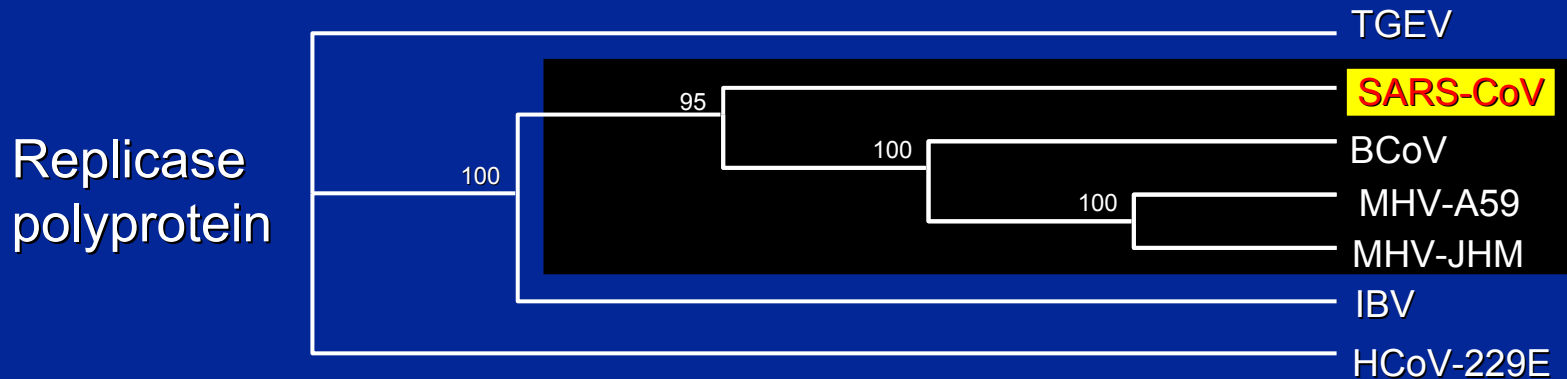
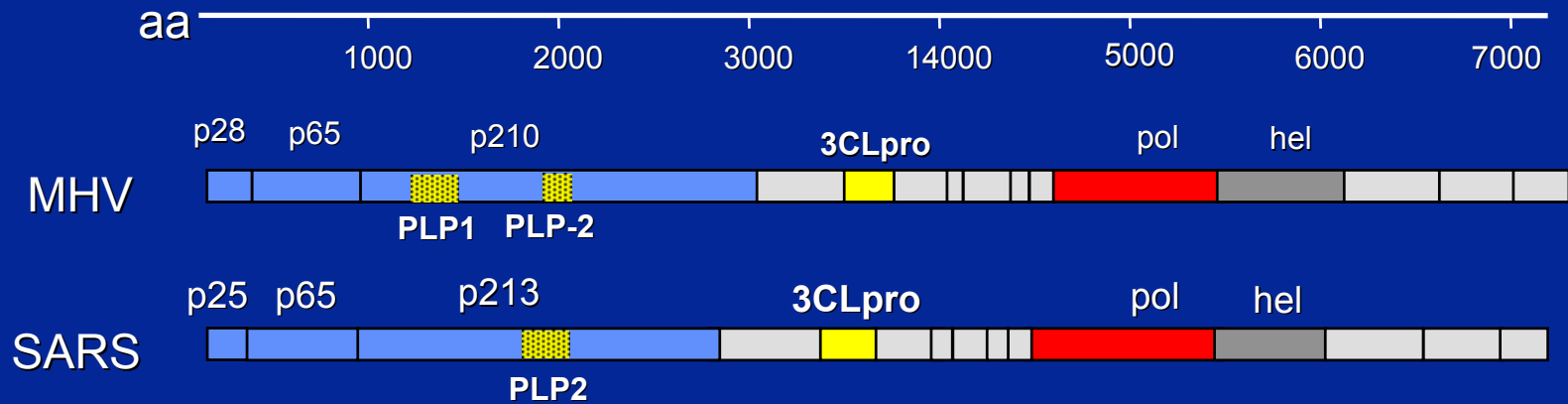
- No human disease
- New models for detection and global public health intervention with emerging infection
- New understanding of coronavirus ecology, evolution, replication, pathogenesis and potential for human disease
- New models for antivirals and vaccines

Phylogeny of Coronavirus Replicase



Method: Neighbor Joining; Bootstrap (100 reps); tie breaking = Systematic
Distance: uncorrected ("p") Gaps distributed proportionally

Phylogeny of Coronavirus Replicase



Coronavirus Biology and Disease: General Themes

- Recurrent / repeat infections
- Prolonged or persistent virus shedding
- Cross species infections in culture and animals
- Rapid adaptation, emergence of new variants
- Changes in diseases and transmission

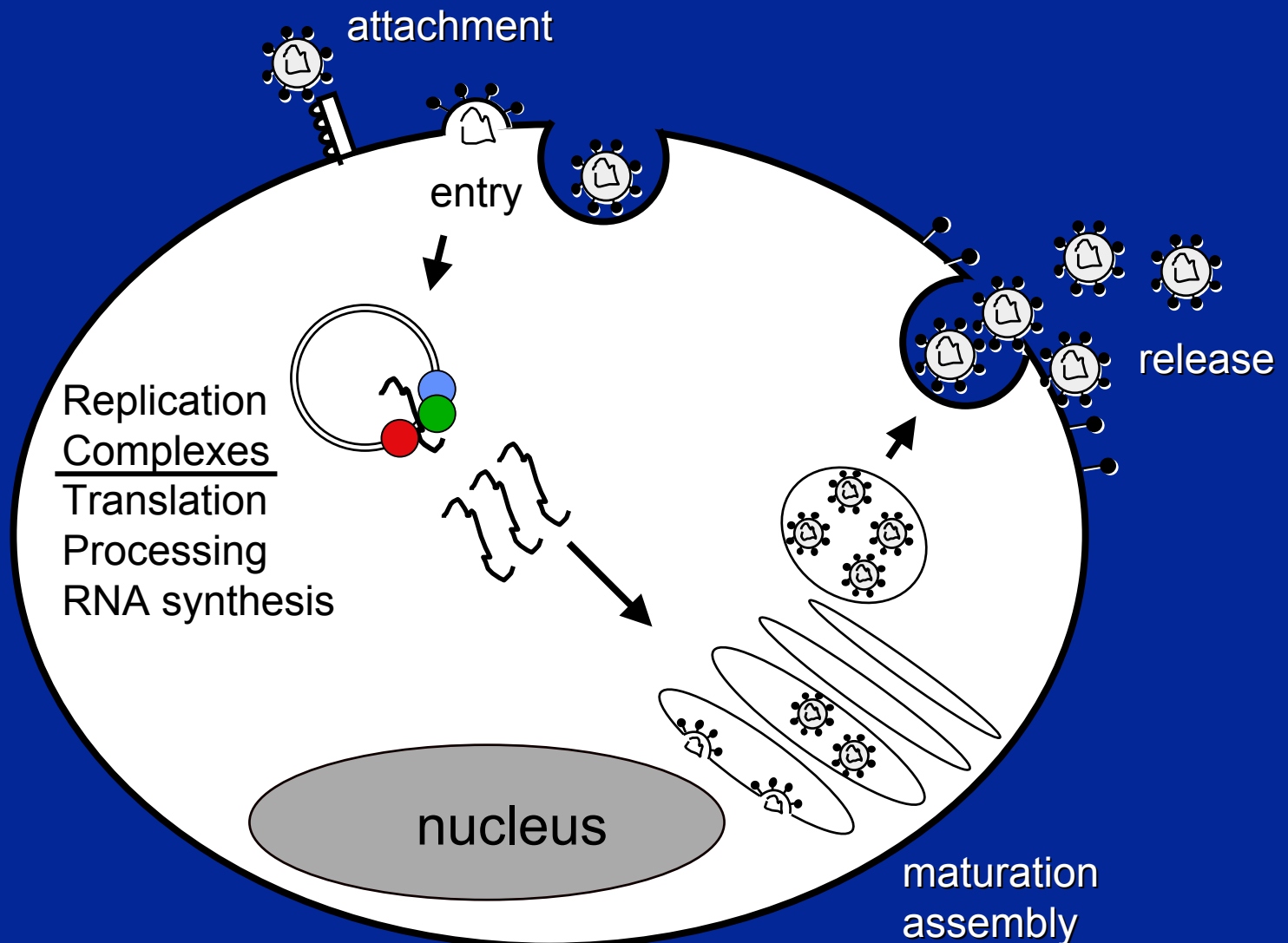
Coronavirus Molecular Biology: General Themes

- Mutation rate: 1 change every 10,000 bases
= 3 changes in every new genome!
- RNA-RNA homologous recombination
- Rapid adaptation, recovery of virulence
- Genome tolerates deletions, mutations, substitutions

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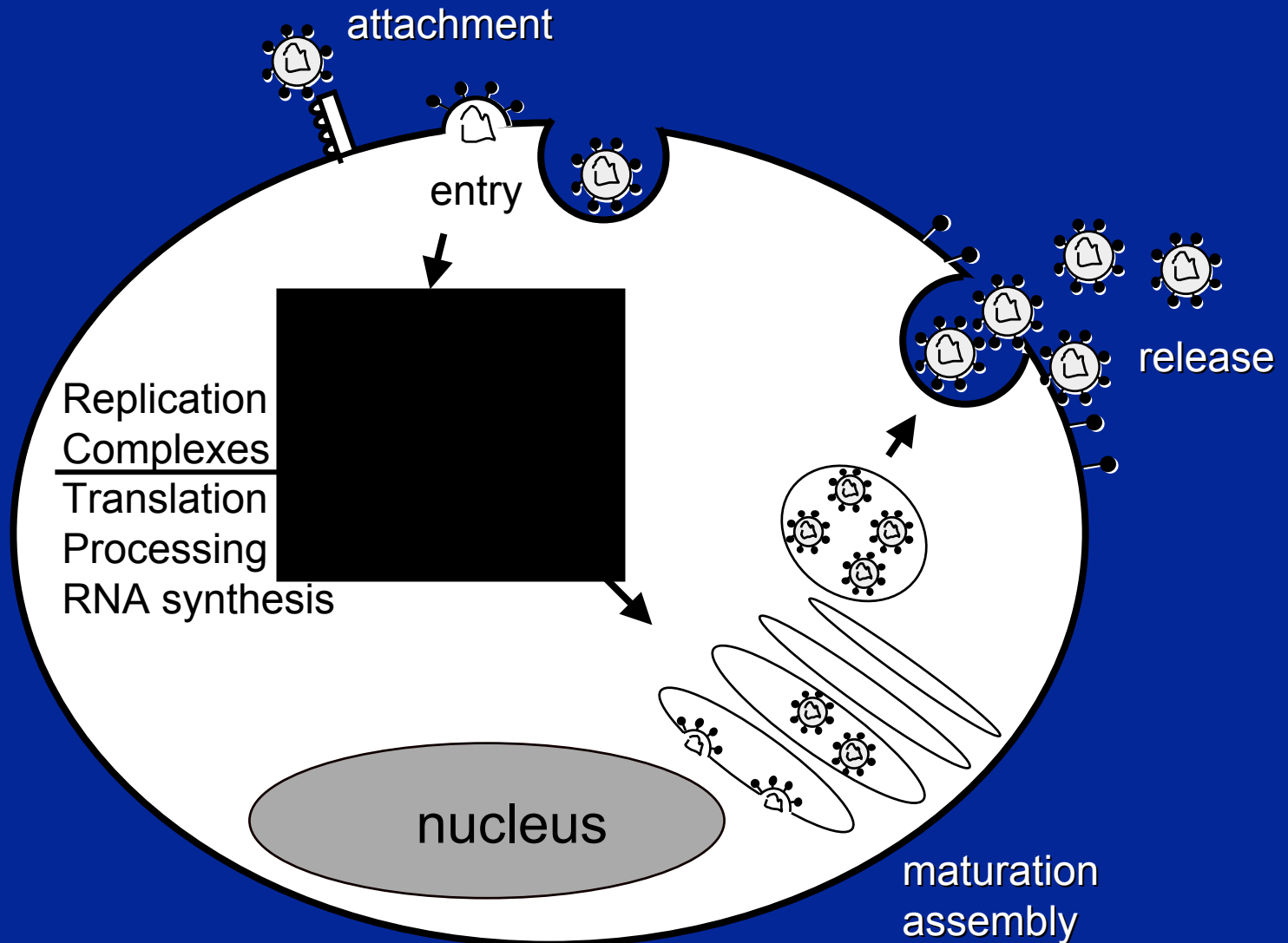
Coronavirus Life Cycle



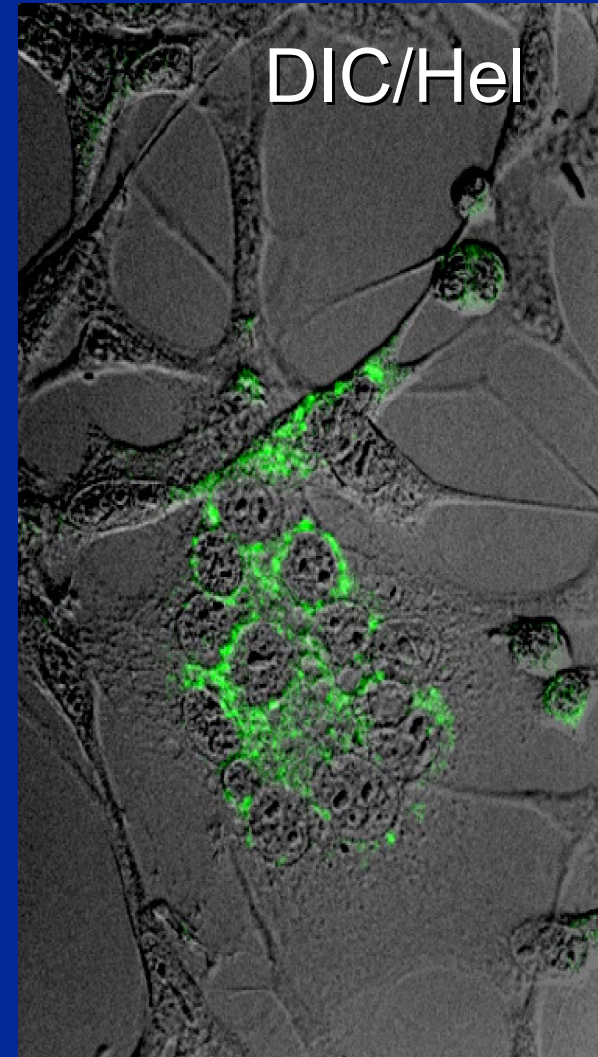
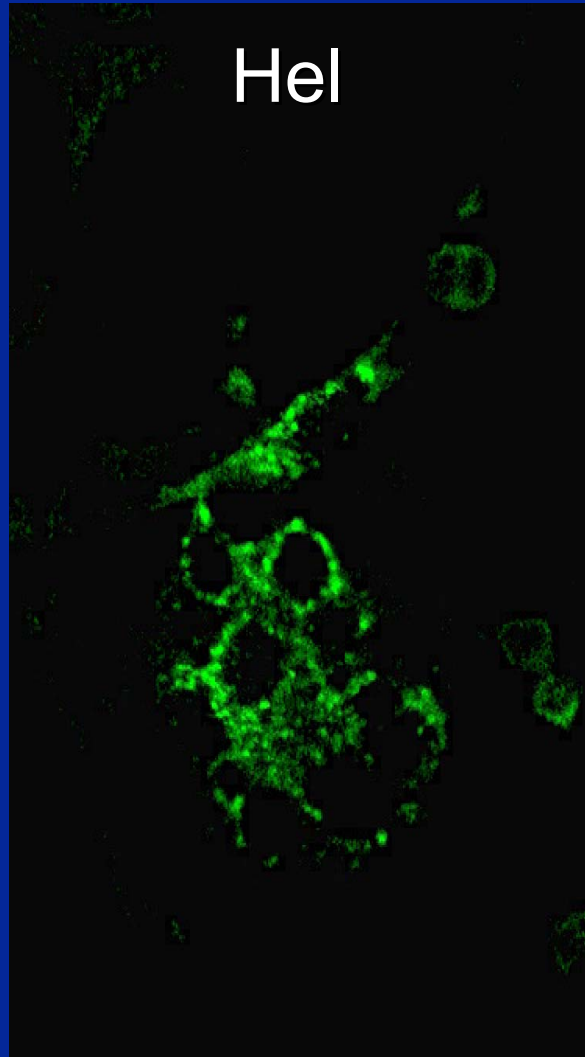
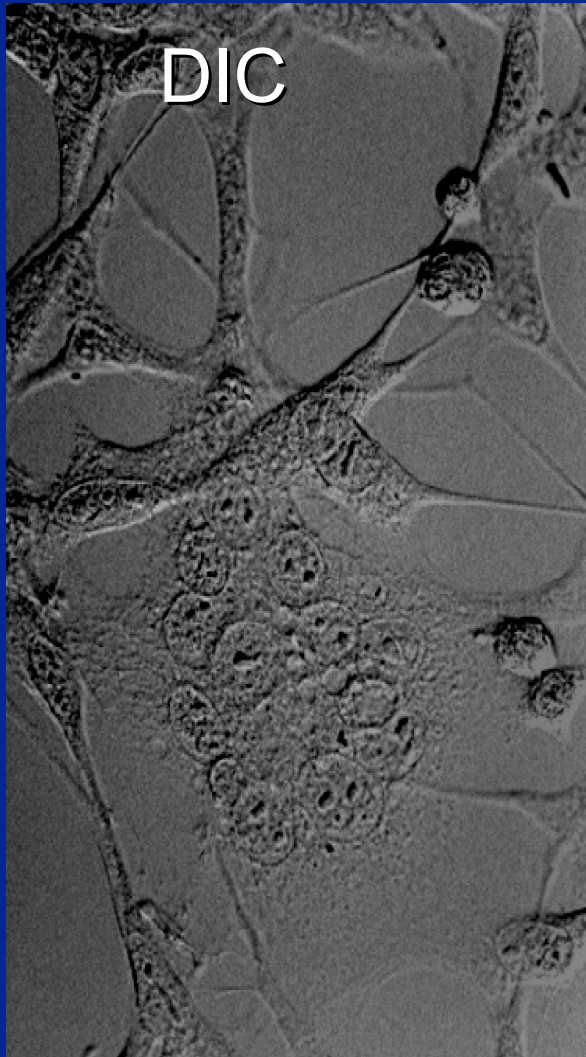


01:22:32

Coronavirus Life Cycle



MHV-A59 infected DBT cells



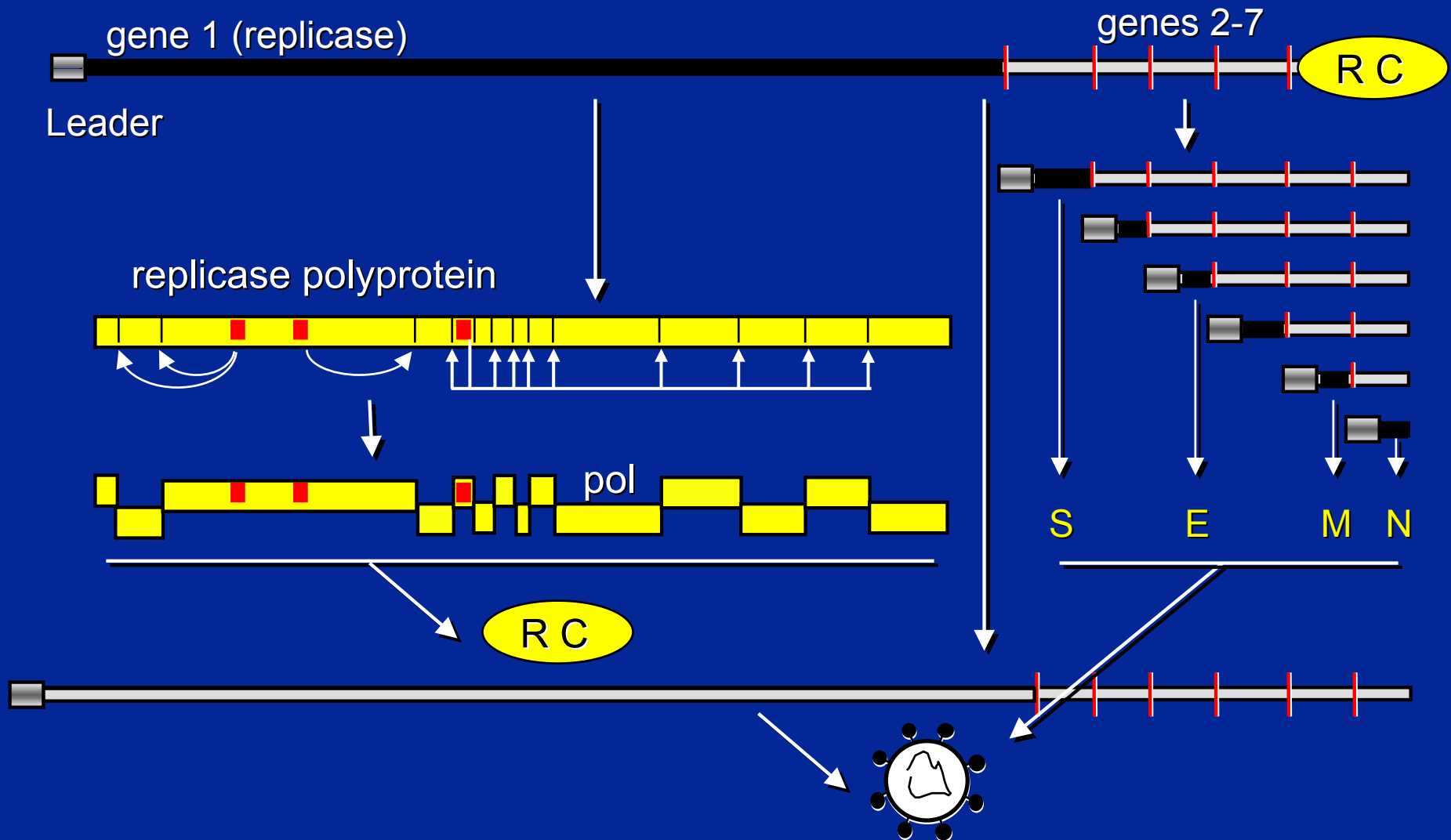
Replicase Proteins



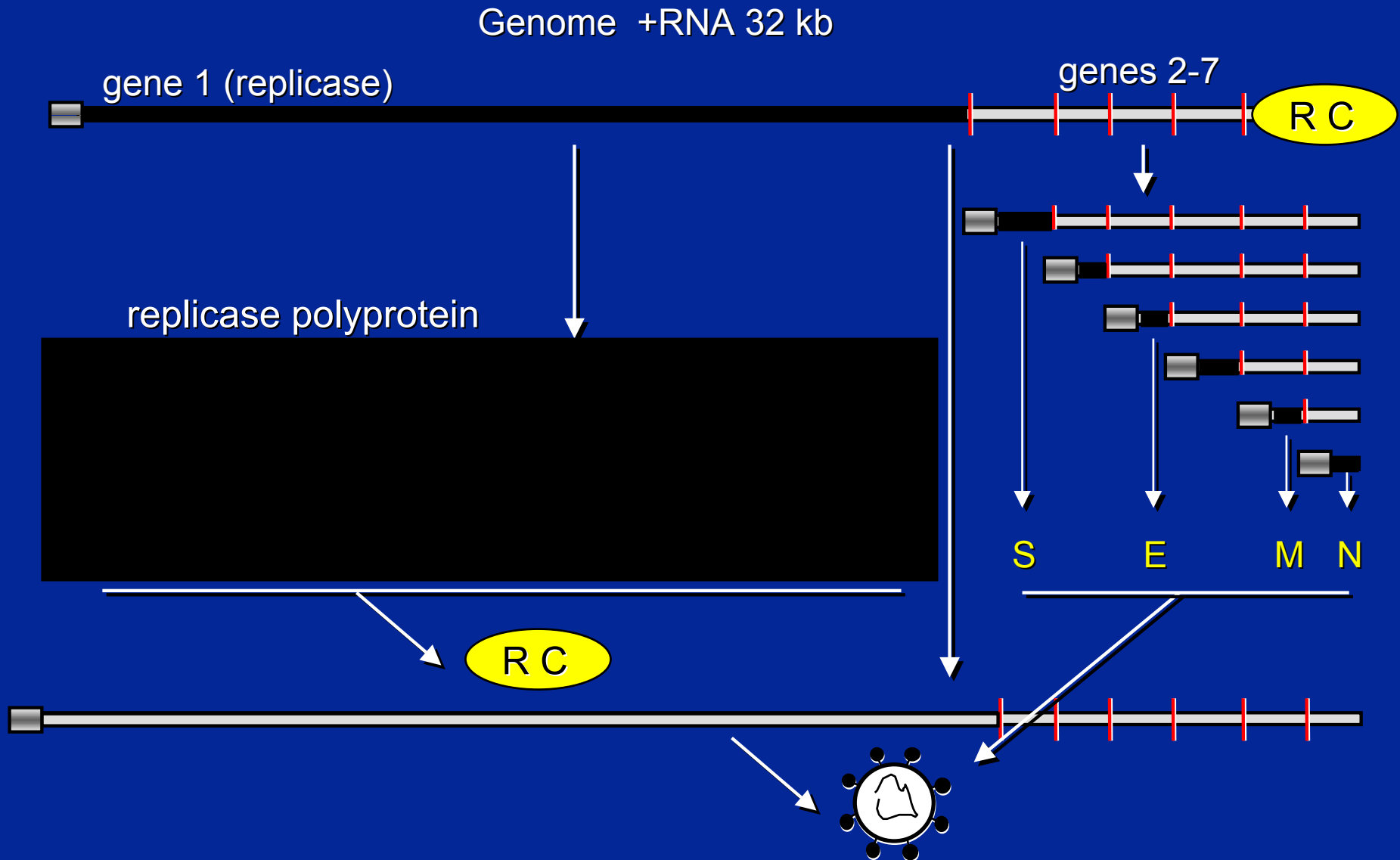
QuickTime™ and a Graphics decompressor are needed to see this picture.

Coronavirus genome and replication

Genome + RNA 32 kb

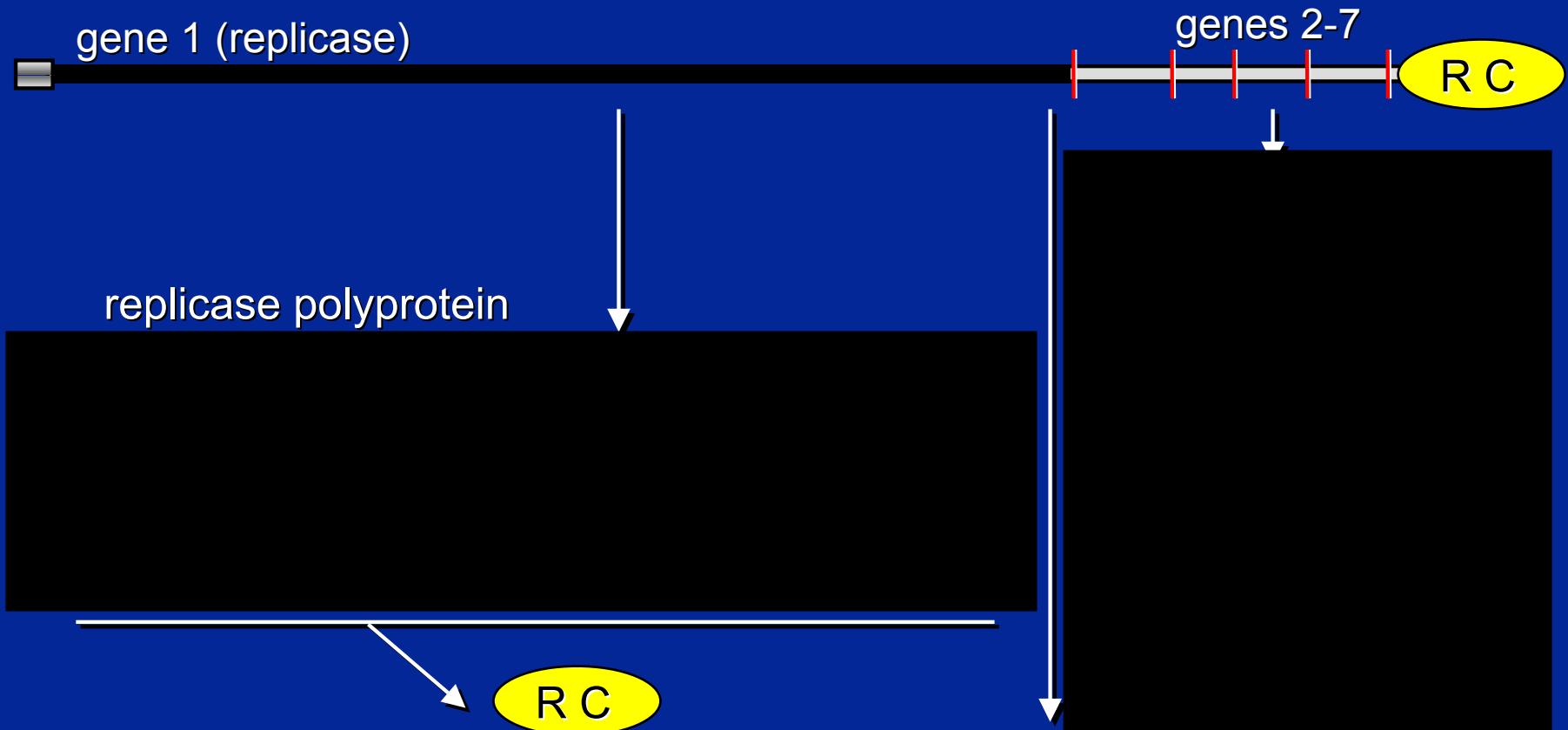


Coronavirus genome and replication

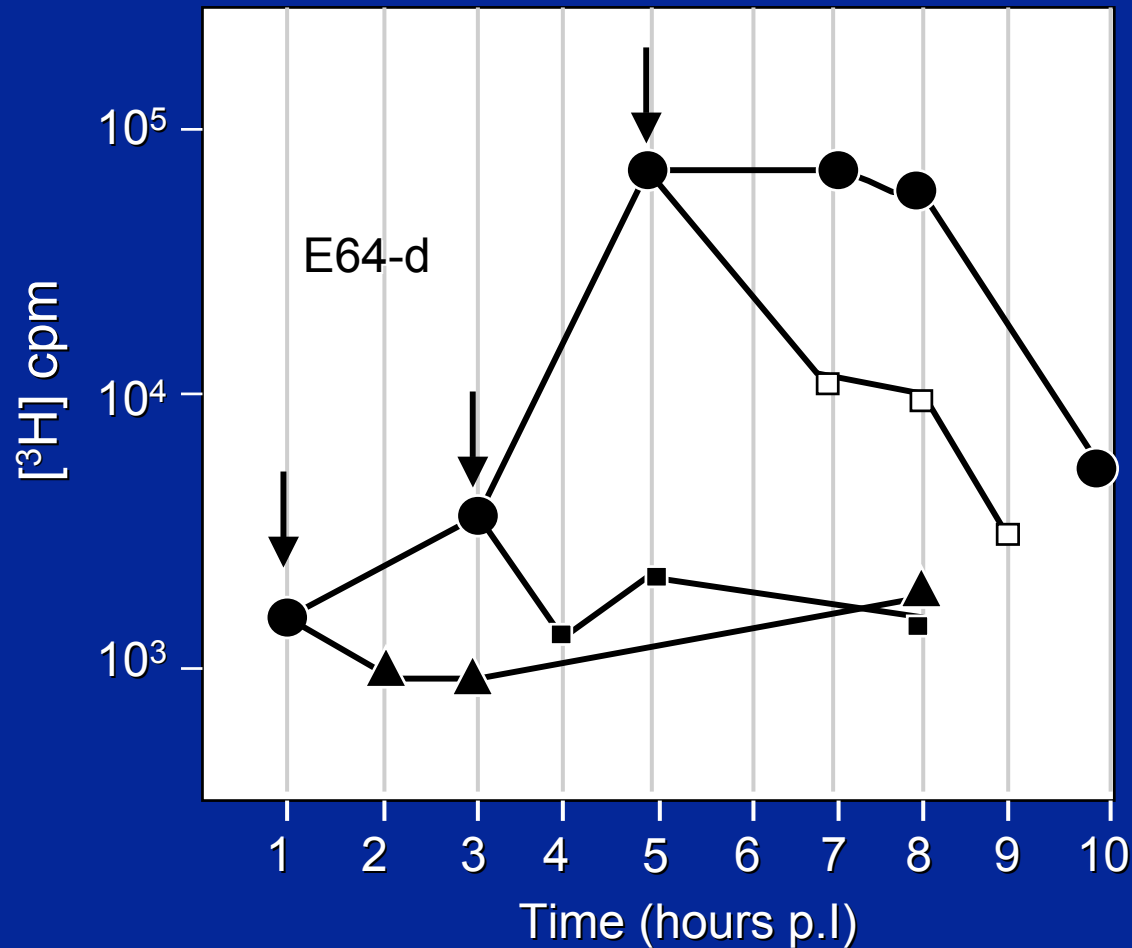


Coronavirus genome and replication

Genome +RNA 32 kb



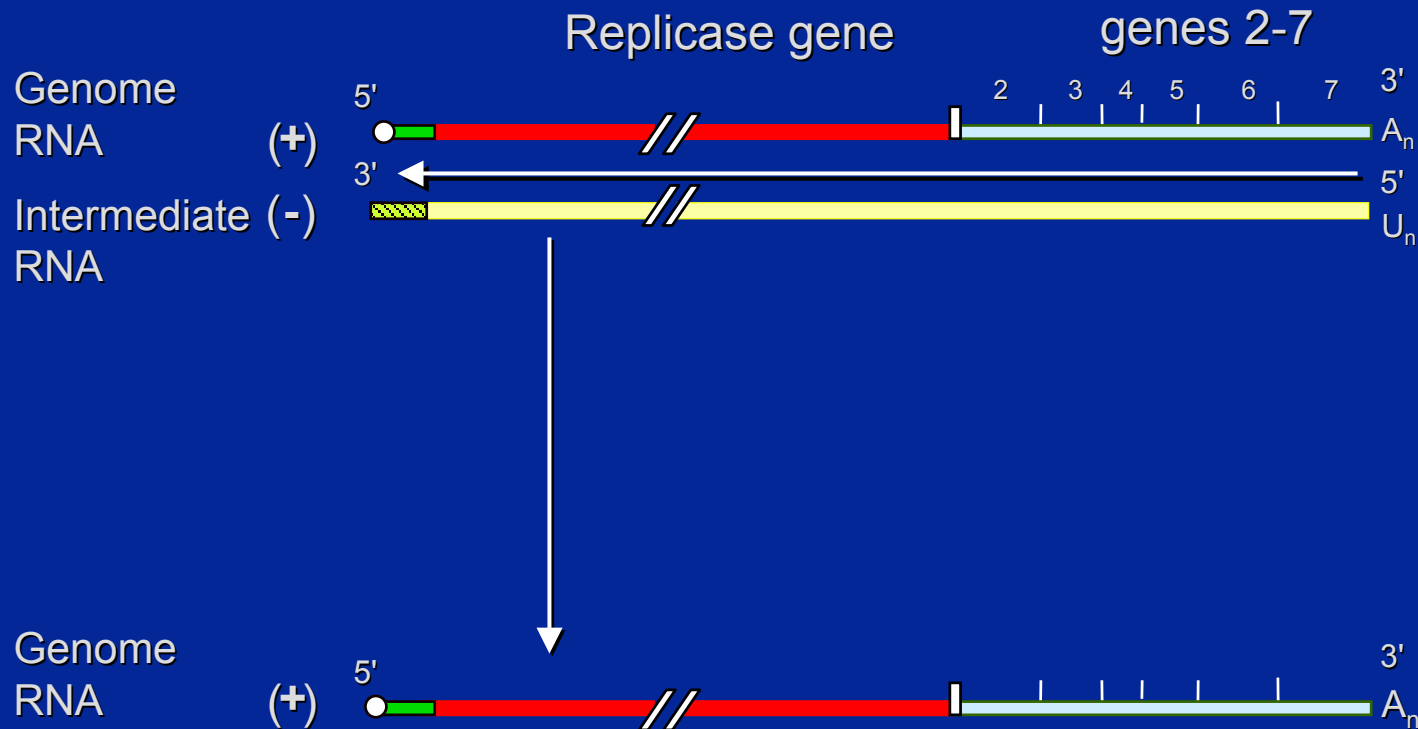
Proteinase inhibitors result in shutoff of viral RNA synthesis at any time during MHV infection



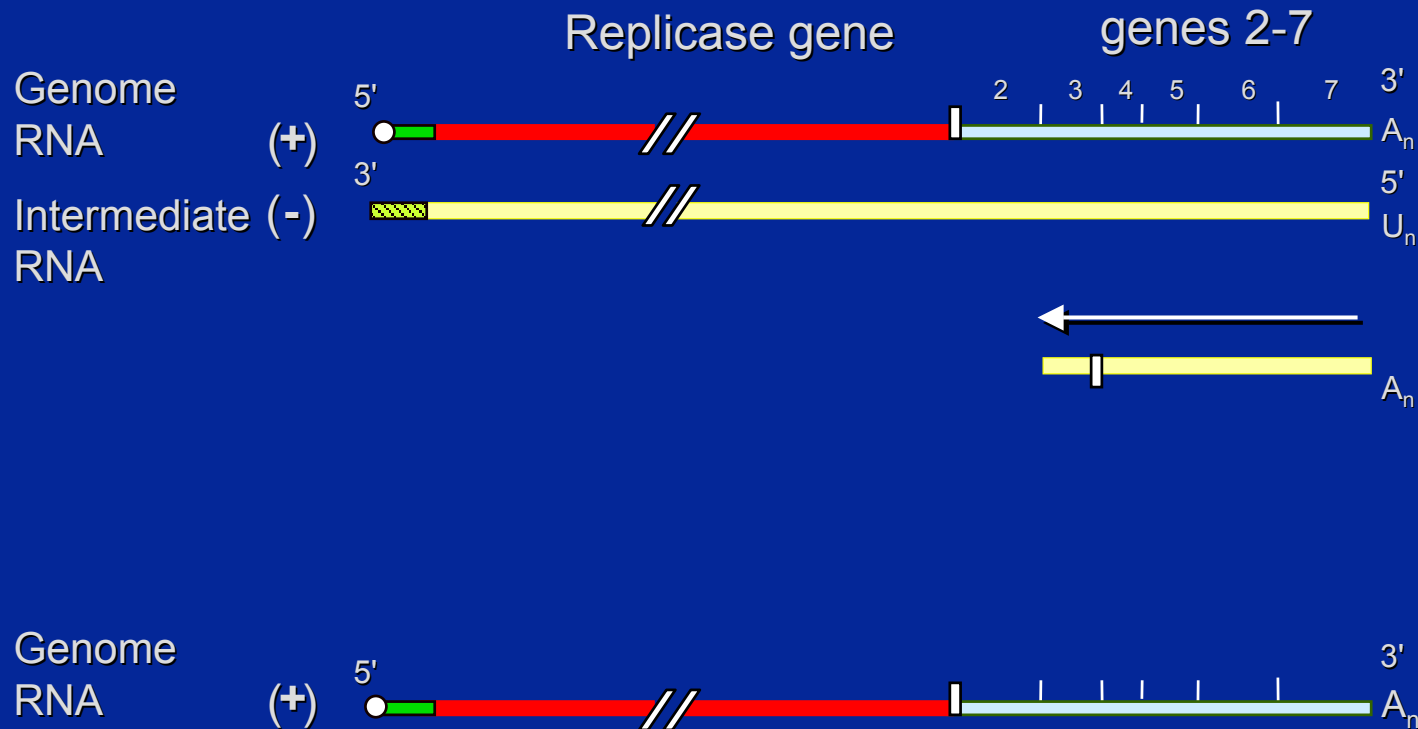
Coronavirus Transcription and Replication



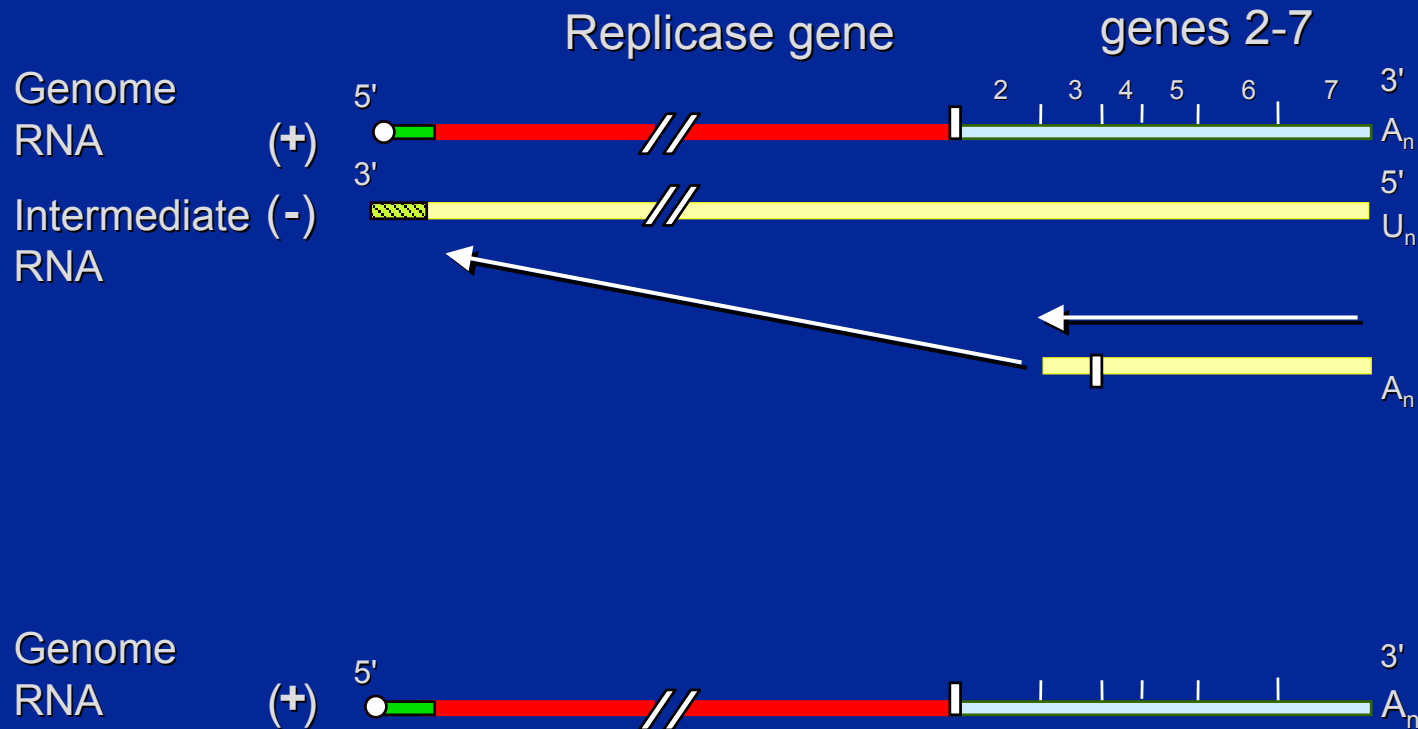
Coronavirus Transcription and Replication



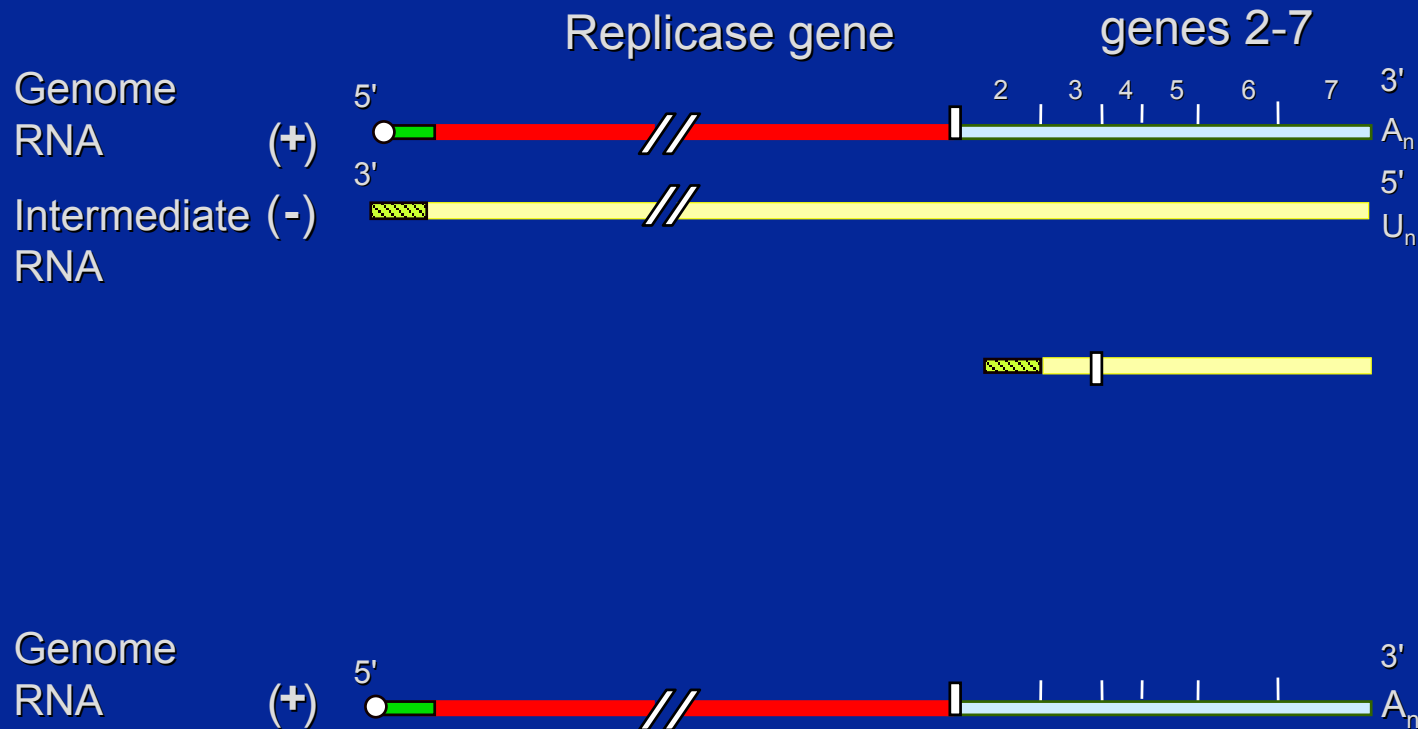
Coronavirus Transcription and Replication



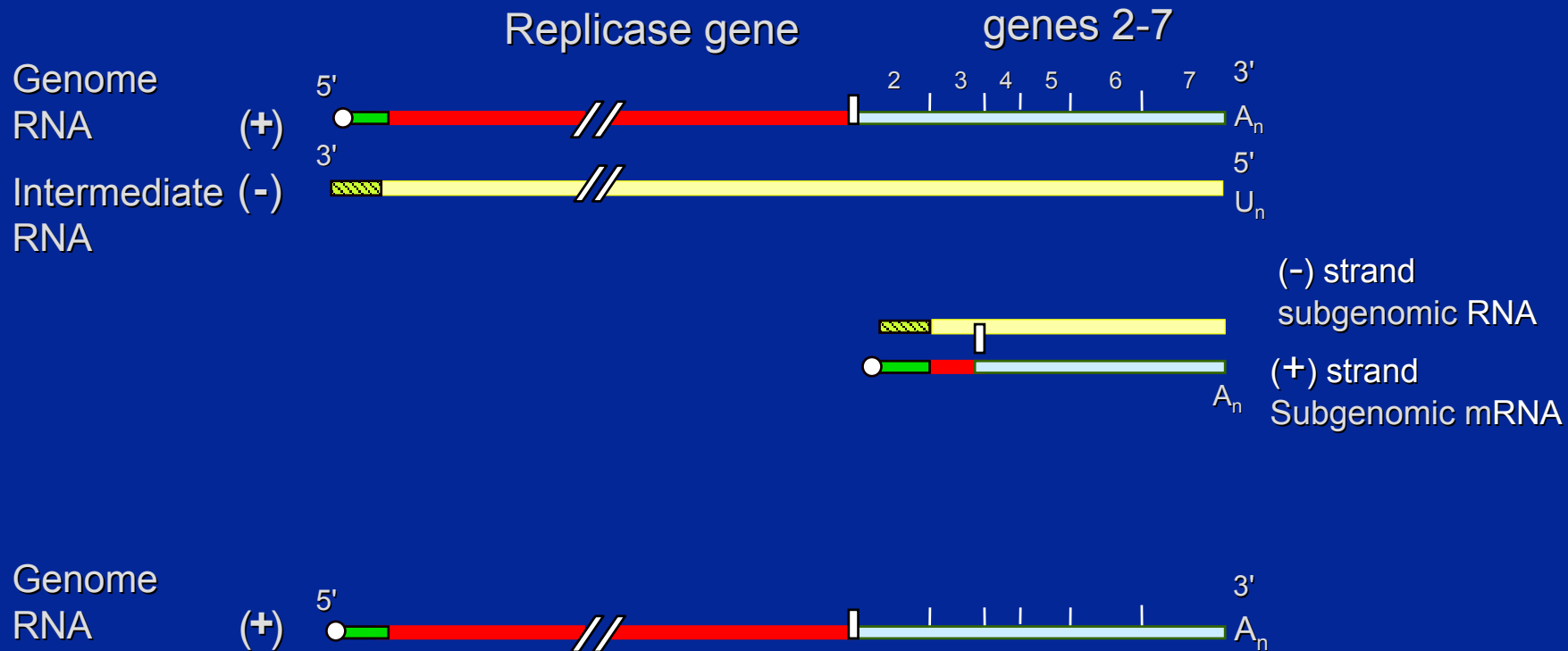
Coronavirus Transcription and Replication



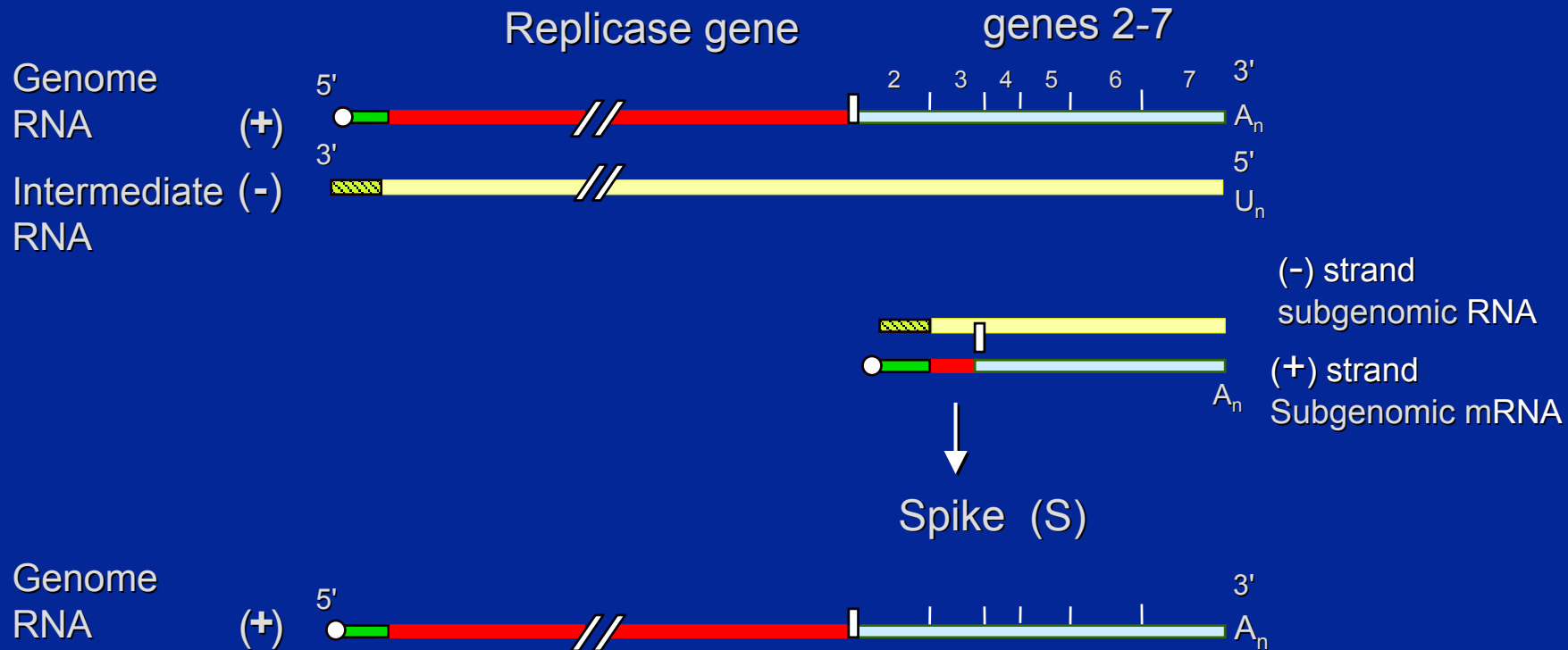
Coronavirus Transcription and Replication



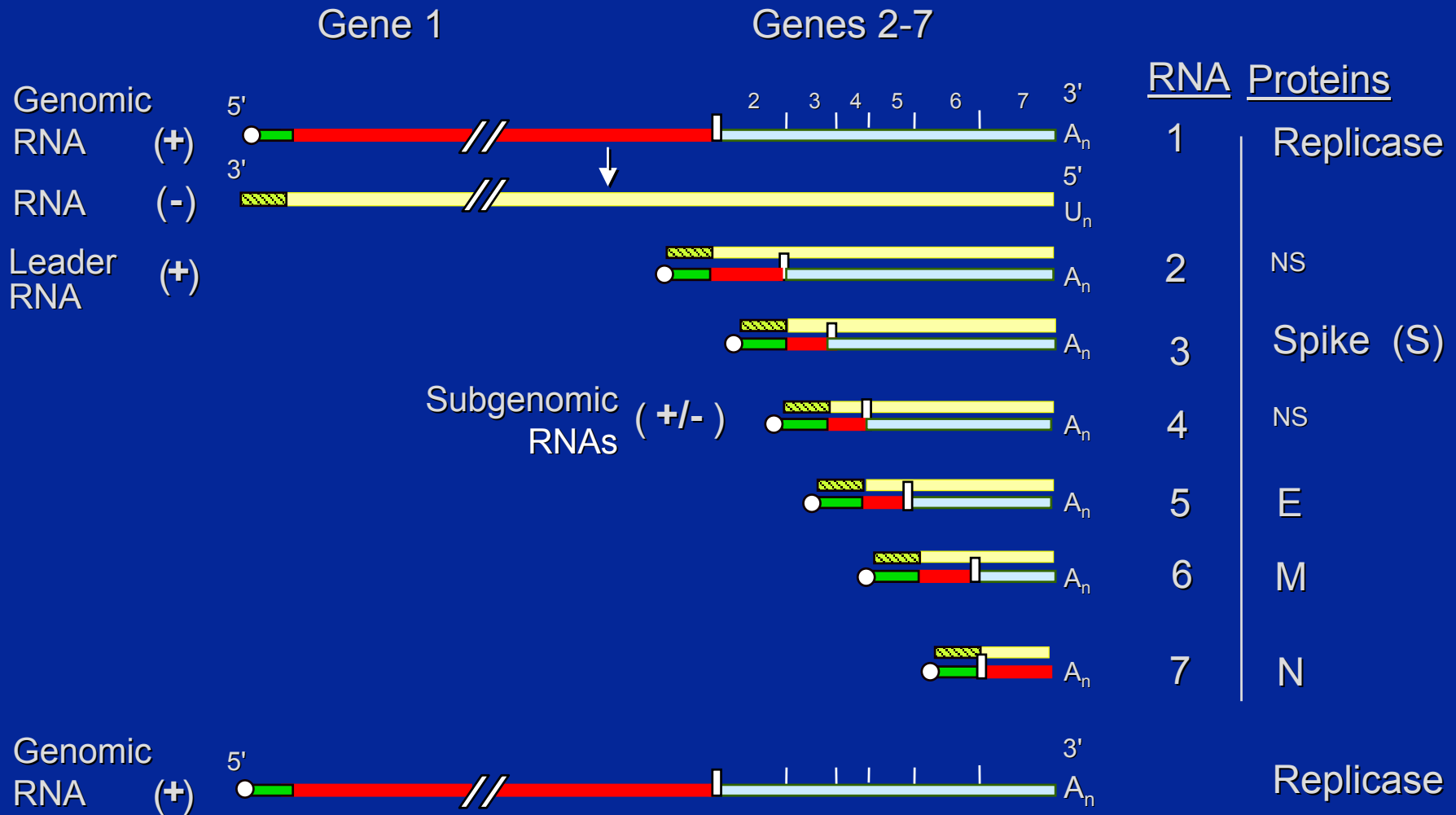
Coronavirus Transcription and Replication



Coronavirus Transcription and Replication



Coronavirus Transcription and Replication



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Coronavirus Genetics: General Themes

- Largest RNA genomes: up to 32 kb ss plus-sense RNA
- “Error-prone” polymerase
- Polymerase template switching and recombination
- Recovery of virulence following deleterious changes
- Mutability and plasticity of genome

Genetic Analysis of Coronaviruses

- Natural variants and mutants

Bovine (BCoV-E): severe diarrhea \longrightarrow BCoV-R: pneumonia

Porcine (TGEV): severe diarrhea \longrightarrow PRCoV: respiratory

Feline (FIPV): peritonitis \longrightarrow FECoV: diarrhea

??? \longrightarrow SARS-CoV: SARS

Mouse (MHV-A59): hepatotropic \longleftrightarrow MHV-JHM: neurotropic

- Temperature Sensitive Mutants

- Variations in replication and pathogenesis

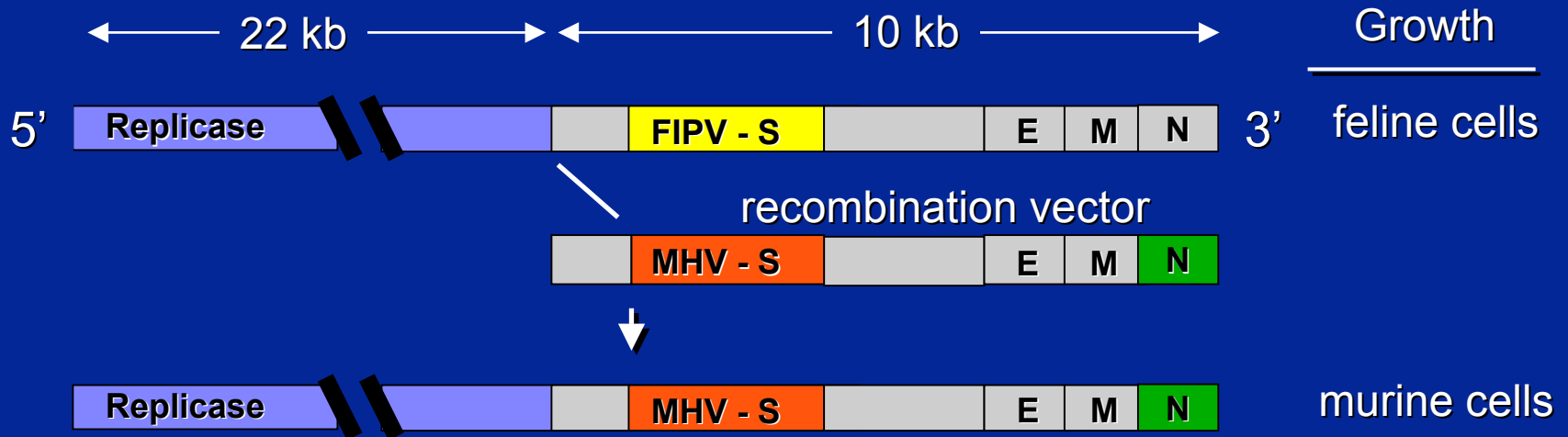
- Vaccine strains

- Passage in culture and animals, UV, chemical, cold, cell mixing

Genetic Analysis of Coronaviruses

- Sequencing of viruses, variants, and mutants
- Reverse genetics: introduction of mutations into genome
 - Targeted recombination
 - Infectious cDNA cloning

Targeted Recombination



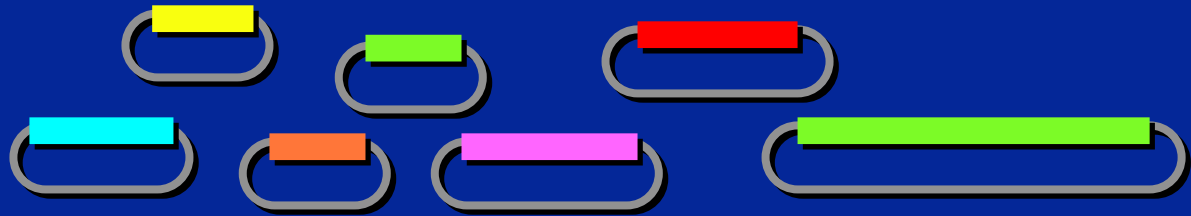
- Takes advantage of high rate of homologous recombination:
- Powerful selection for mutations- growth on specific cells
- Applicable to 3' 10 kb of genome
- Includes all structural and “non-replicase” nonstructural genes
- Has been well utilized for studies of S, M, N and E genes



Genome + strand RNA (32 kb)



RT / PCR
cDNA clones



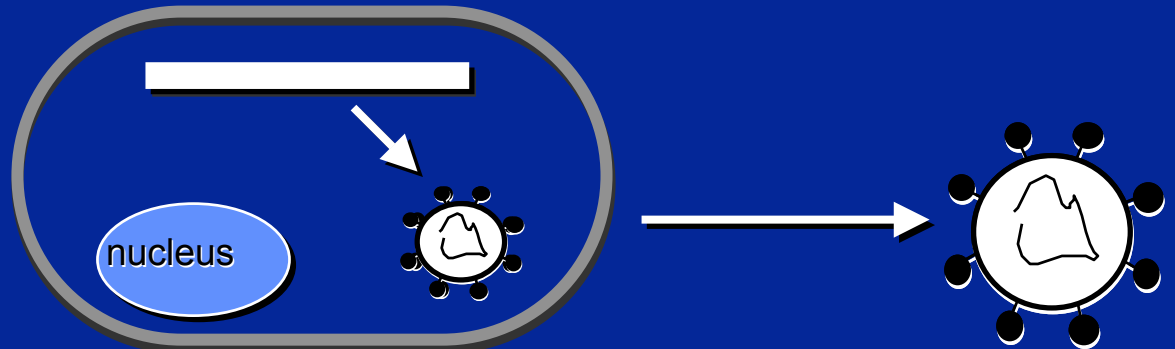
In vitro ligation



Transcribe
+ strand RNA



Transfect cells

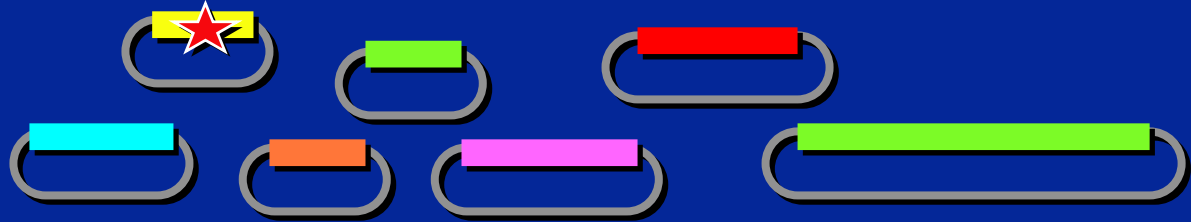




Genome + strand RNA (32 kb)



RT / PCR
cDNA clones



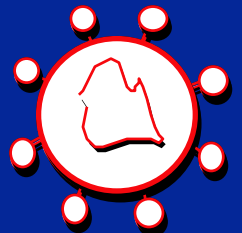
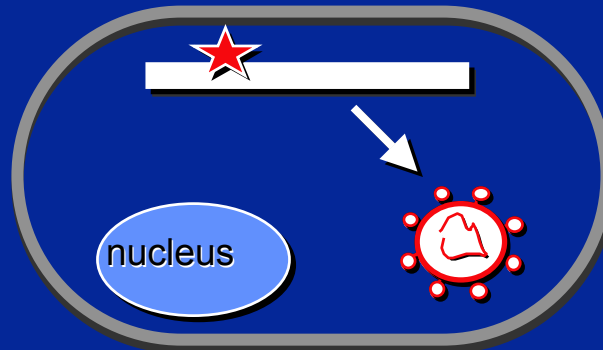
In vitro ligation



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Transfect cells

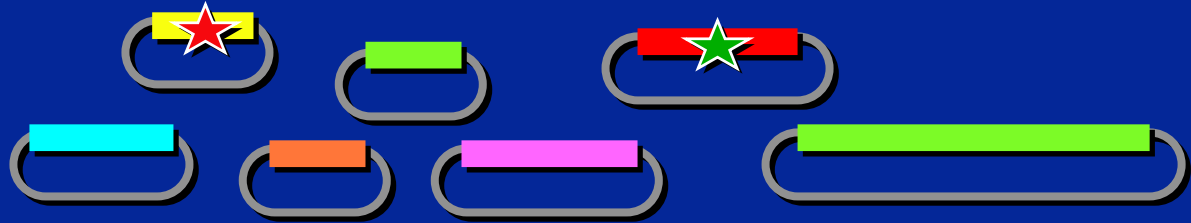




Genome + strand RNA (32 kb)



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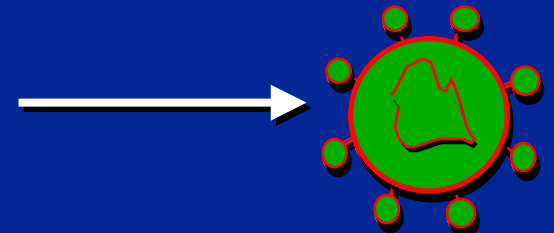
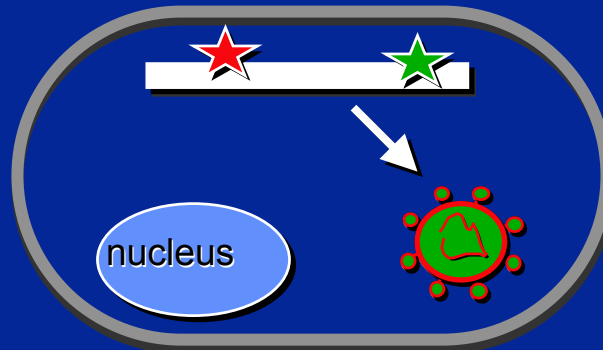
In vitro ligation



Transcribe
+ strand RNA



Transfect cells



Coronavirus Infectious cDNA Cloning

- Infectious clones : MHV, IBV, TGEV, HCoV-229E
 - In vitro assembly, Vaccinia recombinants, BAC
- Applicable to entire genome
- Example of mutations yielding viable viruses
 - Gene deletions, gene duplications
 - Gene substitution (GFP)
 - Gene order rearrangement
 - Replicase polyprotein cleavage site deletion

Application of Molecular Biology and Genetics to SARS-CoV

- Genome organization, proteins, replication strategy likely retained
- Requirements for cell entry, protein expression and processing, RNA synthesis, virus assembly
- Closest relationships to group 2 coronaviruses
- Animal models for SARS-CoV
- Integration of natural variation and direct genetic approaches

Recommendations for Molecular and Genetic Studies of SARS-CoV

- Sequence lab and clinical isolates of human, mammalian, and avian coronaviruses.
- Identify and sequence new mammalian, avian and other coronaviruses
- Longitudinally track, database, and analyze SARS-CoV isolates
- Develop reverse genetic system for SARS-CoV
- Cloning and expression of SARS-CoV proteins and receptors for antibodies functional studies and inhibition
- Define cell biology of SARS-CoV: Replication complexes, mechanisms of cell death